August

Week 1 (01/08/16 - 05/08/16)

R0011-K1362011 and J04500-K1184000 induction and solubility analysis, Endolysin, Endoholin and Holin RFC 25 ligation and transformation.

The goal for this week consisted in the ligation of the parts related to the project in a previously purified TOPO vector in order to transform them into DH5alpha competent cells. Furthermore, it was required the solubility analysis for both selected characterization cassettes.

- MODULE 1: Induction and solubility analysis of J04500+K1184000 cassette.

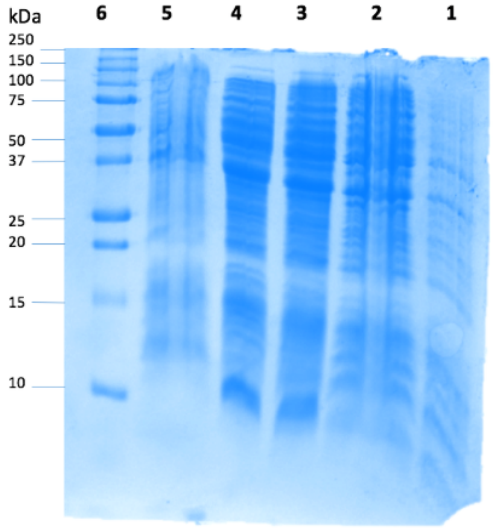

Fig. 1 R0011-K1362011 Induction SDS-PAGE. Lamba lysozyme has an expected weight of 17.83 kDa. Gel at 15% run at 150V for 2 hour and 20 minutes. **1)** Hour 0 (No induction) - 20µL, **2)** Hour 1 - 20µL, **3)** Hour 2 - 20µL, **4)** Hour 3 - 20µL, **5)** BL21 control - 20µL, **6)** Precision Plus Protein Prestained Protein Standard (Bio-Rad) - 5µL.

Even though the gel did not run in the proper manner, stains of proteins can be observed just above the 15 kDa mark which is the expected weight of the desired Lambda lysozyme; these stains are present in the second, third and fourth wells , while in wells 1 and 5 no mark is visible. Despite this result could indicate the presence of the protein, due to the wrong running of the gel, this induction and solubility analysis is not conclusive.

MODULE 2: Transformation

Transformation of synthesis in E. coli DH5alpha competent cells.

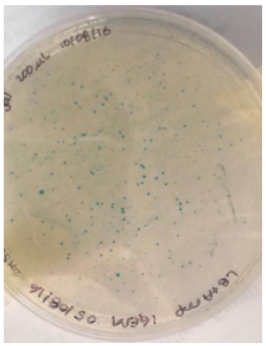

Fig. 2 Transformation of the holin (RFC 25) in DH5alpha competent cells plated on LB+AMP with X-Gal medium.

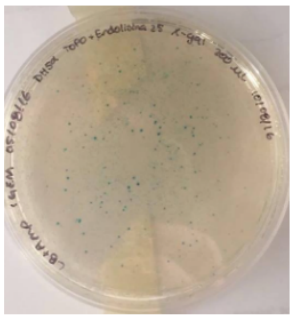

Fig. 3 Transformation of the endolysin (RFC 25) in DH5alpha competent cells plated on LB+AMP with X-Gal medium.

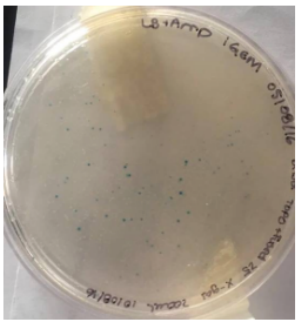

Fig. 4 Transformation of the endoholin (RFC 25) in DH5alpha competent cells plated on LB+AMP with X-Gal medium.

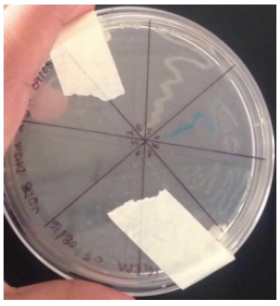

Fig. 5 Transformation of the endolysin (RFC 10, without RBS) DH5alpha competent cells plated on LB+AMP with X-Gal medium.

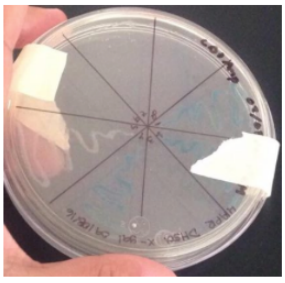

Fig. 6 Transformation of the holin (RFC 10) in DH5alpha competent cells plated on LB+AMP with X-Gal medium.

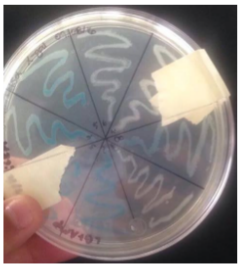

Fig. 7 Transformation of the endolysin (RFC 10, RBS) in DH5alpha competent cells plated on LB+AMP with X-Gal medium.

The growth of white-colored colonies seen in Figures 2, 3 and 4 indicates that some TOPO vectors were successfully ligated to the holin, endolysin and endoholin, respectively; whereas the blue-colored colonies represent that the digested TOPO vector was ligated without the genes of interest.

Even though, blue-colored colonies predominated in all the transformation plates, the white-colored ones were chosen for stria culturing. In figures 5, 6 and 7, blue colonies can be seen, which sometimes happen if the **β**-galactosidase gene takes longer to be expressed, showing light blue-colored colonies that can be confused with the white-colored ones.

Summary:
Solubility and induction analysis are becoming a major concern since we haven't had a single 15% gel run the proper way.
Transformations of the project parts were successful and further stria cultures were cultured. Organization of the project is already being discussed and documented, further documentation of the parts is still required.

Week 4 (22/08/16 - 26/08/16)

Extraction of Endolysin, Endoholin, Holin RFC 25, J0445 and R0011.
Digestion and Characterization of Lambda Lysozyme cassette (R0011-K1362011)

- MODULE 1: Restriction

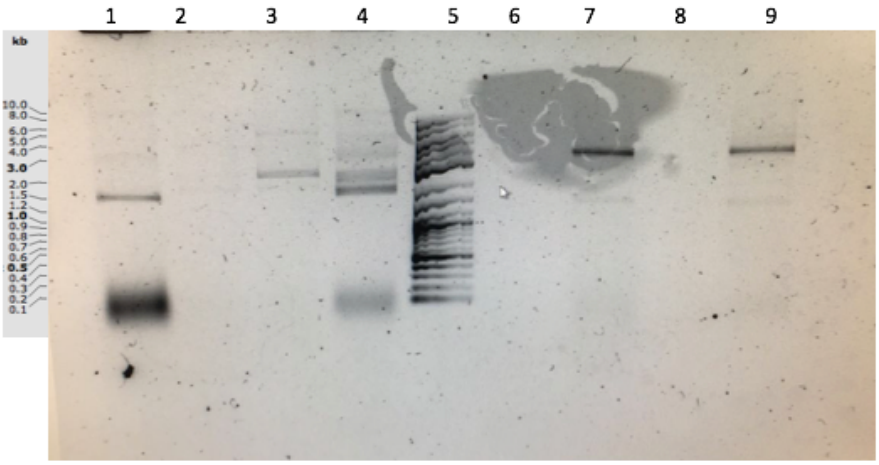

Fig. 3 Plasmid Digestion. Gel at 8% run at 100V for 50min. 1)J04450 plasmid control 5µL + 4µL LB. 2) ZDWZ plasmid control 5µL + 4µL LB. 3) TDSH plasmid control 5µL + 4µL LB. 4) NNQW plasmid control 5µL + 4µL LB. 5) 2 log DNA ladder 2µL + 4µL LB. 6) --- 7) ZDWZ digestion with X+P 40µL + 8µL LB. 8) --- 9) ZDWZ digestion with E+P 40µL + 8µL LB.

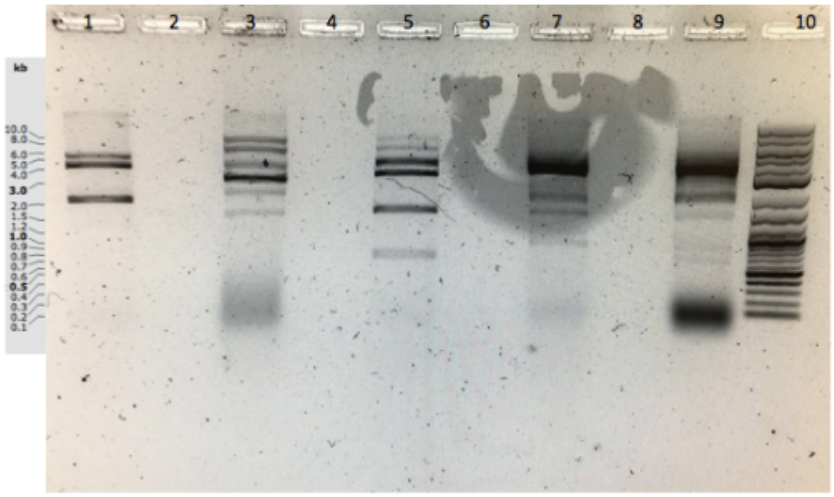

Fig. 4 Plasmid Digestion. Gel al 8% run at 100V for 50min. 1)J04450 digestion with E+P 40µL + 8µL LB. 2) --- 3) TDSH digestion with X+P 40µL + 8µL LB. 4) --- 5)TDSH digestion with E+P 40µL + 8µL LB. 6) --- 7) NNQW digestion with X+P 40µL + 8µL LB. 8) --- 9)NNQW digestion with E+P 40µL + 8µL LB. 10) 2 log DNA ladder 2µL + 4µL LB.

Afterwards restriction was performed (figure 3 and 4) with non-favorable results, since the bands could not be appreciated in a way that would allow their purification; this could have happened because although we used a higher amount of loading dye (8µl) it was not enough for the amount of sample loaded in the gel, they will be performed again using a higher amount of loading dye.

- MODULE 2: Rolambda characterization

Table 3. OD results for the characterization essay.

	Original	Duplicate
	Single Blank	Single Blank
Sample	OD	OD
1	0.877	0.979
2	0.838	0.951
3	0.905	0.879
4	0.903	0.886
5	0.862	0.848
6	0.897	0.803
CONTROL	0.837	

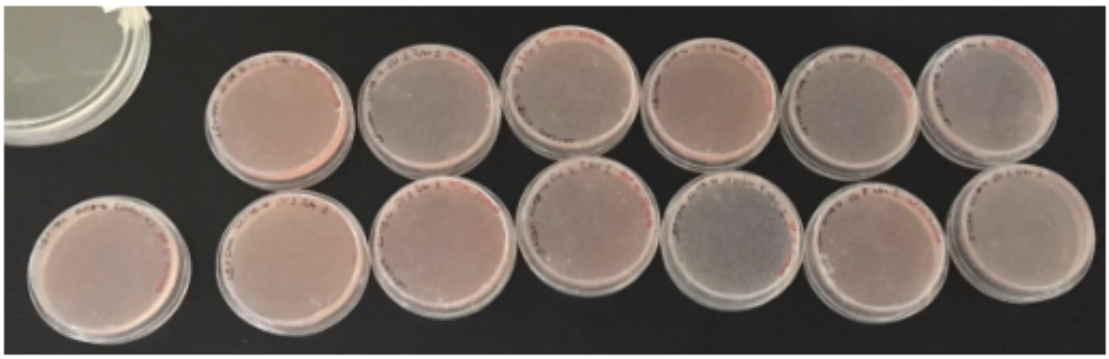

Fig. 5 Plates for each dilution (original and duplicated samples) from alternative 1.

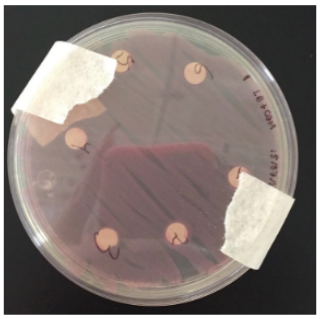

Fig. 6 Antibiogram 1 using the original samples in a LB + CAM medium plated with DH5α + J04450 cells.

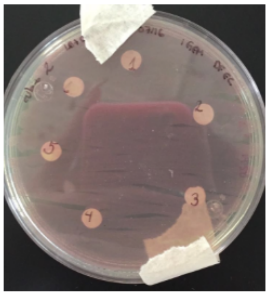

Fig. 7 Antibiogram 2 using the duplicated samples in a LB + CAM medium plated with DH5α + J04450 cells.

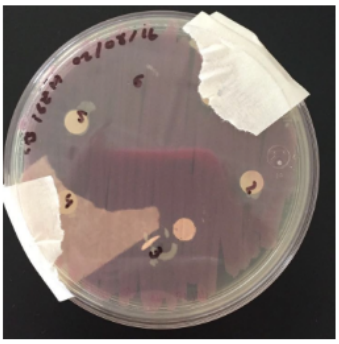

Fig. 8 Antibiogram 3 using the original samples in a LB medium plated with DH5α + J04450 cells.

Regarding the Rolambda characterization, the results from alternative 1 were the expected since the OD was inversely proportional to the amount of protein extract added (table 3); the cellular viability was reduced by adding the protein extract. However, all the dilutions were plated on Petri dishes and the growth was equal in all of them, showing an uniform bacterial lawn but zero colonies (figure 5).

The alternative 2 was unsuccessful since the 3 antibiograms behaved the same regardless there were used both samples (the original and the duplicated ones) and a control without the corresponding antibiotic (figure 6, 7 and 8).

The incongruence among the results of the Petri dishes on both alternatives might be caused by the dilutions' preparation. We strongly suggest to work with different concentrations of pure protein extract diluting it with water, instead of mixing different quantities of DH5α + J04450 cells and protein extract.

Summary:
Characterization of the R0011-K1362011 cassette, was performed changing the volumes of the proteic extract and maintaining constant volume. As the readings performed by the spectrophotometer were varying in a small amount, and the dilutions and antibiograms were not conclusive, we propose to increase the concentrations, with a range of at least half of the ones used (50 ul).
The plasmid extractions of the project were successful, digestions for the parts were performed. The digestions showed non favorable results, as the bands could not be cut because they weren't separated enough. Another restriction will be made.